Abutilon theophrasti: Wild Host For Three Fungal Parasites of Soybean

P. R. Hepperly, B. L. Kirkpatrick, and J. B. Sinclair

Assistant professor of Plant Pathology, Department of Crop Protection, University of Puerto Rico, Mayaguez 00708; former post-doctorate candidate, Department of Agronomy, University of Illinois; and Professor of Plant Pathology, Department of Plant Pathology, University of Illinois, Urbana 61801, respectively.

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ABSTRACT

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Three pathogens of soybean, *Phomopsis sojae* (*Diaporthe phaseolorum* var. *sojae*), *Colletotrichum dematium* var. *truncata*, and *C. gloeosporioides* (*Glomerella cingulata*) were isolated from velvetleaf, a common weed in soybean fields. Lethal stem canker induced by *P. sojae* developed in 1–2% of velvetleaf plants examined in Champaign County and the pathogen was recovered from 25–65% of the stem sections when plants were ≥4 wk old. Recoveries of *C. gloeosporioides* and *C. dematium* var. *truncata* ranged from 0 to 35% and 0 to 85%, respectively. Inoculation of Amsoy 71 soybean seedlings with *C. gloeosporioides* induced leaf cupping and veinal necrosis on expanding leaves. Velvetleaf isolates of *C. dematium* var. *truncata* and *P. sojae* usually were more virulent on soybean pods than were soybean isolates. Isolates of *C. gloeosporioides* from soybean and velvetleaf were avirulent on soybean pods. Inoculation of soybean pods with *Colletotrichum* spp. reduced *P. sojae* in seed. Velvetleaf isolates of *C. dematium* var. *truncata* and *P. sojae* had greater growth rate than soybean isolates on soybean pod and seed extract agar and all isolates regardless of host source grew more rapidly on velvetleaf stem extract agar.

Additional key words: anthracnose, pod and stem blight, seed quality, weed hosts.

Pod and stem blight is a widely distributed disease of soybean (*Glycine max* [L.] Merr.) induced by *Phomopsis sojae* Leh. (*Diaporthe phaseolorum* [Cke. & Ell.] var. *sojae* Wehm.) (9). The fungus may be seedborne and causes reduced germination and seed quality (6). *P. sojae* persists for long periods in plant debris and most workers emphasize the importance of this inoculum source (2,5,7). Weed hosts of the pathogen that may be sources of inoculum have received little attention.

Two distinct *Colletotrichum* spp. are found on soybean (11): *C. dematium* var. *truncata* which induces seed quality losses (12) and *Glomerella glycines* (*C. glycines*), which originally was considered to be the sexual stage of *C. dematium* var. *truncata* (10). Based on isolations from various hosts, over 1,000 species of *Colletotrichum* have been described. Von Arx (15) has suggested that there are only six definable species of *Colletotrichum* based on morphological characteristics.

MATERIALS AND METHODS

Occurrence of fungi on velvetleaf and soybean. In mid-September 1977, two surveys were made to determine: the extent and cause of velvetleaf stem canker and the occurrence of fungi on velvetleaf stems. Two-hundred velvetleaf plants from soybean fields on the Plant Pathology Research Farm at Urbana were examined for stem cankers. Tissues with cankers were excised, surface disinfected by immersion in 0.5% NaOCl (10% Clorox) for 4 min, and plated on potato-dextrose agar (PDA) (Difco, Difco Laboratories, Detroit, MI 48232) for 1–2 wk at 25 C.

To assay for the occurrence of fungi on mature healthy velvetleaf stems, four 3-cm pieces were cut from each of 10 plants collected from five soybean fields at the Plant Pathology Research Center. Stem sections were incubated for 72 hr in a moist chamber at 95% RH and 25 C. Fruiting fungi were identified to genus.

In 1978 Champaign County was surveyed for the occurrence of *P. sojae* on velvetleaf stems. Ten mutually equidistant locations were selected throughout the county. Within 2 km of each location, 15–20 velvetleaf plants growing in soybean fields were harvested. The seventh internode of each plant was removed, surface disinfested, plated on PDA, and then incubated for 1–2 wk at 25 C.

A study of mycoflora of velvetleaf and soybean plants growing in the same field was made in a field planted with velvetleaf and soybean (cultivar Amsoy 71) on the Agronomy South Farm in late May 1978. Soybean emerged on 6 June and velvetleaf on 7 June. Velvetleaf seedlings were tagged as they emerged at 2-wk intervals. Ten velvetleaf plants from each emergence period were assayed when 9 wk old. The first, third, and seventh internodes of each species were surface disinfested and assayed on PDA as previously described.

One hundred velvetleaf plants were harvested in 1977 and 1978. Each year seeds were bulked and random samples of 200 seed were surface-disinfested and assayed on PDA.

Pathogenicity studies. One-week-old cultures of *P. sojae* from velvetleaf stem cankers were used as inoculum sources for pathogenicity studies. Surface mycelium was scraped into 15-ml of sterile distilled water, ground with a mortar and pestle, and passed through a 149-μm (100-mesh) screen. Four-week-old soybean (cultivar Amsoy 71) seedlings were atomized with the inoculum mixture at a rate of 100 ml of suspension per plant. Inoculated plants were placed in a greenhouse, individually covered with plastic bags for 2 days, and then observed daily for symptom development. After 30 days, the plants were assayed for presence of the fungus . Plants sprayed with atomized sterile distilled water served as controls. There were four plants in each sprayed and nonsprayed group. Amsoy 71 soybean seeds were surface dininfested as previously described for stem tissue and 1 drop of the inoculum was applied to each of 200 seeds. Noninoculated seeds served as controls. All seeds were plated on PDA and germination and radicle lengths were recorded after 2 wk at 25 C.

The pathogenicity of *C. gloeosporioides* from velvetleaf and soybean was determined by using the procedures described above for isolating *P. sojae* from stem cankers.

A detached-pod technique was used to determine the pathogenicity of various isolates of *C. dematium* var. *truncata*, *C. gloeosporioides*, and *P. sojae* from latent stem infections in soybean or velvetleaf collected from the same field. Field-grown

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Amsoy 71 soybean pods with green full-sized seed were harvested, disinfested, and placed for 1 wk in sterile moist chambers at 95% RH and 25 C. Prior to incubation groups of the pods either were inoculated with *P. sojae,* inoculated with *C. dematium* var. *truncata,* inoculated with *C. gloeosporioides,* or not inoculated. Isolates to be tested were selected randomly from among those obtained from latently infected field plants. Six isolates, three each of velvetleaf and soybean, were inoculated with each fungus (*P. sojae* and *C. dematium* var. *truncata*). Four isolates, two from each host, were used for *C. gloeosporioides.* Inoculum was prepared by adding 15 ml of sterile water to each 9-cm-diameter fungus culture plate and scraping of the surface growth from 2-wk-old PDA cultures. One drop of inoculum was applied on the surface of pods over the site of each developed seed. Pod lesions were recorded. All pods were dissected and seeds were assayed for infections and germination. A completely randomized design was used with 10 pods per replication and each treatment being replicated two or more times. Number of infected pods, pod lesion frequency, seed germination, and the incidence of seedborne *P. sojae* were expressed in relation to the noninoculated control. Recoveries of *Colletrotrichum* spp. from seed was expressed as a gross percentage.

Growth on velvetleaf and soybean extract agars. The six isolates for each of *C. dematium* var. *truncata* and *P. sojae* used for pod pathogenicity tests were also tested for in vitro growth. Media were prepared from 4-wk-old velvetleaf stems (velvetleaf extract agar [VEA]) and from soybean (cultivar Williams) pods with green full-sized seed (soybean extract agar [SEA]). Plant materials were ground 5 min in a Waring Blendor and the homogenate was filtered through four layers of cheesecloth and passed through two layers of Whatman No. 4 filter paper. Three-hundred milliliters of each filtrate was added to 700 ml of water containing 15 g of agar. The media were autoclaved for 15 min and 25 ml was poured into each 9-cm-diameter culture plate. A plug (5 mm diameter) of agar with mycelium from 2-wk-old cultures of each isolate were placed at the center of each test plate (two plates per isolate) and colony diameter was measured after 3 and 4 days at 25 C.

TABLE 1. Pathogenicity of soybean seedborne and velvetleaf canker isolates of *Phomopsis sojae* on cultivar Wells soybean seed

Treatment	Germination (%)[a]	Radicle length (mm)[a]
Noninoculated	98 w[b]	10.8 x
Soybean seedborne 1	0 z	1.5 z
Soybean seedborne 2	0 z	1.7 z
Soybean seedborne 3	76 x	4.8 y
Velvetleaf 1	50 y	3.3 y
Velvetleaf 2	72 x	3.5 y

[a]Percentages germination and radicle length were determined after incubation of Wells soybean seeds on potato-dextrose agar for 2 wk at 25 C. Each mean represent four 50-seed replications.
[b]Means followed by the same letter are significantly different according to Duncan's multiple range test at $P = 0.01$.

TABLE 2. Prevalence of fungi during November 1977 on stem sections of mature velvetleaf (*Abutilon theophrasti*) after incubation in a moist chamber

Fungus species	Occurence[a] (%)
Phomopsis sojae	70
Fusarium spp.	23
Colletotrichum gloeosporioides (*Glomerella cingulata*)	21
Colletotrichum dematium var. *truncata*	12
Glomerella cingulata (*Colletotrichum gloeosporioides*)	11
Macrophomina phaseolina	8
Nigrospora sp.	5
Alternaria sp.	4
Epicoccum sp.	3
Gonatobotrys sp.	1

[a]Stem pieces (200 3-cm stem sections from 50 velvetleaf plants) were held in moist chambers for 72 hr before identification of fungi.

RESULTS

Stem canker was found on velvetleaf plants during 1977, but not in 1978. The incidence of velvetleaf stem canker ranged from 1 to 2% (mean 1.25%) in four fields examined in 1977. Scattered plants died prematurely in early September. Lesions typically were centered at the 3rd, 4th, or 5th node, the dead foliage was retained, and cankered tissue always yielded *P. sojae.*

P. sojae from velvetleaf stem cankers infected both soybean and velvetleaf seedlings in the greenhouse. Isolates from velvetleaf were less pathogenic to soybean seeds than were those from soybean seed (Table 1). Velvetleaf stem canker isolates differed culturally from soybean seed isolates of *P. sojae*; they produced caespitose groups of pycnidia after 1–2 wk and perithecia after 3–4 wk on PDA, while those from soybean seed produced stroma in concentric rings and neither pycnidia nor perithecia were produced after 1 mo on PDA.

C. gloeosporioides was found on mature stems of velvetleaf and on leaves of velvetleaf and soybean. Field infections of velvetleaf were associated with a common leafspot, but no symptoms were associated with soybean infection in the field. In cross-inoculation studies of two isolates from each host, all isolates infected both hosts. Cupping and veinal necrosis on leaves of soybean developed if leaves were expanding when inoculated. Symptoms were evident 5–7 days after inoculation.

A number of fungi were associated with mature velvetleaf stems (Table 2). Over one-half of all stems assayed had *P. sojae* with alpha and/or beta spores. The other most commonly encountered fungi were: *C. gloeosporioides, C. dematium* var. *truncata,* a *Fusarium* sp., and *Macrophomina phaseolina.*

Latent infection of velvetleaf and soybean stems by *P. sojae, C.*

TABLE 3. Fungi isolated from velvetleaf (*Abutilon theophrasti*) and soybean (*Glycine max*) stem sections in 1978

Host	Date of field emergence	Age of plant (wk)	Recovery[a] (%) *Phomopsis sojae*	*Colletotrichum dematium*	*Colletotrichum gloeosporioides*
Velvetleaf	7 June	2	0[b]	0	0
	7 June	4	33	86	3
	7 June	6	27	53	3
	7 June	8	35	55	0
	22 June	2	30	80	0
	22 June	12	55	15	35
Soybean	1 June	9	25[c]	50	5

[a]Stem sections of both velvetleaf and soybeans were sectioned at the first, third, and seventh internode, surface disinfested, and incubated on sterile potato-dextrose agar for 1 wk at 25 C.
[b]Mean of isolations from 10 velvetleaf plants.
[c]Mean of isolations from 20 soybean plants.

dematium var. *truncata,* and *C. gloeosporioides* was common in field plants (Table 3). None of these fungi was isolated from more than 1% of the velvetleaf seeds that were assayed. No infection by these fungi was found in the earliest-emerging group of velvetleaf seedlings, but on later-emerging plants, these fungi always were detected on 2-wk-old plants. *C. dematium* var. *truncata* colonized the vast majority of stem sections early, but the incidence appeared to decline as the season progressed. *P. sojae* infected 25–35% of stem sections throughout the season and the incidence exceeded 50% at the season's end. The incidence of *C. gloeosporioides* was low during most of the season, but rose noticeably at the end. Rates of recovery of these three fungi were similar for both soybean and velvetleaf from the same field. Stem infection by these fungi never exceeded 10% on velvetleaf plants growing in an abandoned field never cropped to corn or soybeans. In survey of 10 soybean fields scattered througout Champaign County *P. sojae* was isolated from 35 to 65% of plants collected in mid-September (Table 4).

TABLE 4. Percentage *Phomopsis sojae* recovery from the seventh stem internode of velvetleaf (*Abutilon theophrasti*) at 10 soybean field sites surveyed in Champaign County, IL, in September 1978

Site number[a]	Plants (no.)	*P. sojae* isolated (%)
1	15	40
2	24	50
3	20	60
4	20	65
5	15	27
6	15	33
7	20	35
8	20	30
9	16	62
10	20	40
		$\bar{X} = 44.9$

[a]To survey the county, 10 equal-sized areas were designated; within 2 km from the center of each area, a soybean field sampling site was chosen and 15 to 20 velvetleaf plants were selected at random from each site.

All isolates used in pathogenicity tests on soybean pods originated from latent infection of soybean and velvetleaf stems. Pathogenicity of *P. sojae* isolated from soybean overlapped that of velvetleaf isolates (Table 5). Generally, isolates from velvetleaf were more pathogenic on soybean pods than were those from soybean. No cultural differences were noted regardless of host origin. All isolates produced concentric rings of stromatic tissue on PDA. Soybean and velvetleaf isolates of *C. dematium* var. *truncata* were similarly pathogenic to pods. *C. gloeosporioides* was not pathogenic to soybean pods. Both of these *Colletotrichum* spp. reduced the seedborne incidence of *P. sojae* (Table 5).

Depending on host origin, growth differences were found for both *P. sojae* and *C. dematium* var. *truncata* isolates on SEA. Soybean isolates of each fungus grew more slowly on SEA than did velvetleaf isolates. An isolate of *P. sojae* from soybean, which would not grow on SEA, was avirulent on soybean pods. Differences of growth of isolates based on their host origin were not evident on VEA.

DISCUSSION

This is the first report of velvetleaf stem canker. Kmetz et al (8) found that nonstromatic isolates of *P. sojae* from soybean produced stem cankers and were less pathogenic on soybean seeds than were stromatic isolates. Similar results were obtained with stem canker isolates from velvetleaf.

In the past, speciation of *Phomopsis* and *Colletotrichum* was based largely on host. Two morphologically distinct *Colleto-*

TABLE 5. The pathogenicity of *Colletotrichum dematium, Colletotrichum gloeosporioides,* and *Phomopsis sojae* from soybean (*Glycine max*) and velvetleaf (*Abutilon therophrasti*) on soybean pods and seeds[a] and the growth of these fungi on soybean extract agar (SEA) and velvetleaf extract agar (VEA)[b]

Fungus	Origin	Isolate no.	Germination[c]	Pod lesions[c]	Infected pods[c]	Seedborne *Phomopsis sojae*[c]	Seedborne *Colletotrichum* species (%)	Growth[d] SEA	Growth[d] VEA
P. sojae	Soybean	3	108[d]	96	95	92	0	0.0	32.0
		5	50	160	150	160	0	8.0	36.7
		6	22	323	226	323	0	5.3	36.5
	Velvetleaf	10	78	251	166	272	0	13.7	39.8
		11	31	300	214	313	0	16.7	34.5
		12	67	236	178	236	0	9.2	34.8
C. dematium	Soybean	2	136	204	179	44	34	3.3	20.8
		3	167	68	95	24	18	4.2	21.3
		5	140	80	107	68	11	7.0	20.8
	Velvetleaf	10	97	308	213	47	72	10.5	19.8
		11	143	317	214	72	64	9.8	17.0
		12	131	188	154	72	32	9.3	18.8
C. gloeosporioides	Soybean	1	90	64	72	60	4	...[f]	...
		2	110	118	107	62	5	...	...
	Velvetleaf	11	105	78	72	14	18	...	...
		12	113	146	142	72	3	...	...
FLSD[g] ($P = 0.05$)			28	60	23	40	15	1.5	1.2

[a]Surface-disinfested Amsoy 71 soybean pods were used at green full-bean stage for inoculation. To prepare inoculum, 15 ml of water was added to 9-cm-diameter culture plates containing a 2-wk-old culture of the test fungus on potato dextrose agar. Surface mycelium was scraped free and the mix of mycelium and water was ground with mortar and pestle. Three drops of inoculum were applied by medicine dropper for every pod tested.

[b]For preparing media 4-wk-old velvetleaf stems or soybean pods with full-sized green beans were used. Materials were homogenized and filtered. Three hundred milliliters of each filtrate were added to 700 ml of water containing 15 g of agar.

[c]Values expressed as percentage of noninoculated control having 38% germination, 25% pod lesions, 42% infected pods, and 25% seed infection by *P. sojae.*

[d]Increase of colony diameter (millimeters) growing from a 5-mm-diameter plug for 3 days at 25 C on soybean or velvetleaf extract agars.

[e]Means are based on two replications each of 10 soybean pods.

[f]No data.

[g]Fischer's least significant difference, $P = 0.05$.

trichum spp. are found on soybean (13). *C. dematium* var. *truncata* can be distinguished by relatively large (2.5 to 4.0 × 16 to 30 μm) falcate conidia. *C. gloeosporioides* produces smaller (4 to 8 × 9 to 24 μm) cylindrical conidia. No sexual stage was discovered for *C. dematium* var. *trunicata;* however, *C. gloeosporioides* commonly produces the *G. cingulata* stage. Historically, considerable confusion centers about the differentiation of the two *Colletotrichum* species on soybeans. Lehman and Wolf (10) reported the *G. cingulata* stage of *C. gloeosporioides* and recognized it as the sexual stage of *C. dematium* var. *truncata.* They gave the fungus the designation by host: *C. glycines (G. glycines).* We find *C. glycines* as described for soybean and *C. malvarum* described on velvetleaf to be synonymous with *C. gloeosporioides. C. gloeosporioides* is reported to have a wide host range (14).

Weeds may serve as alternate hosts for pathogens that affect soybean seeds. Weeds were associated with production of low quality soybean seed in Brazil (4), Mississippi (1), and in Illinois (B. L. Kirkpatrick, *unpublished*). In Brazil, weed infestation was positively correlated with soybean seed infection rates of *C. dematium* var. *truncata, Fusarium semitectum,* and *P. sojae.* In Mississippi, cocklebur infestation was associated with increased numbers of damaged seeds and reduced soybean seed grade (1). We found that both *C. dematium* var. *truncata* and *P. sojae* from velvetleaf were highly pathogenic to soybean seeds and pods. The possible role of weeds as inoculum sources for seed-decay fungi should not be underestimated.

C. gloeosporioides has been suggested for the biological control of jointvetch in rice (3). Our studies indicate that this species appears to infect many of its hosts latently. Screening a wide range of plants for latent infection would be advisable when examining possible biological control agents.

Both *C. dematium* var. *truncata* and *C. gloesporioides* inoculated on soybean pods reduced the percentage of seed that developed *P. sojae* infections. Further work is needed to determine how this antagonism is effected and its significance, if any.

LITERATURE CITED

1. ANDERSON, J. M., and C. G. McWHORTER. 1976. The economics of common cocklebur control in soybean production. Weed Sci. 24:397-400.
2. ATHOW, K. L., and R. M. CALDWELL. 1954. A comparative study of Diaporthe stem canker and pod and stem blight of soybean. Phytopathology 44:319-325.
3. DANIEL, J. T., G. E. TEMPLETON, R. J. SMITH, Jr., and W. T. FOX. 1973. Biological control of northern jointvetch in rice with an endemic fungal disease. Weed Sci. 21:303-307.
4. DHINGRA, O. D., and J. F. da SILVA. 1978. Effect of weed control on the internally seedborne fungi in soybean seeds. Plant Dis. Rep. 62:513-516.
5. GERDEMAN, J. W. 1954. The association of *Diaporthe phaseolorum* var. *sojae* with root and basal stem rot of soybean. Plant Dis. Rep. 38:742-743.
6. HEPPERLY, P. R., and J. B. SINCLAIR. 1978. Quality losses in *Phomopsis*-infected soybean seed. Phytopathology 68:1684-1687.
7. HILDEBRAND, A. A. 1954. Observations on the occurrence of the stem canker and pod and stem blight fungi on mature stems of soybean. Plant Dis. Rep. 38:640-646.
8. KMETZ, K., C. W. ELLET, and A. F. SCHMITTHENNER. 1977. Isolation of seedborne *Diaprthe phaseolorum* and *Phomopsis* from immature soybean plants. Plant Dis. Rep. 58:978-982.
9. LEHMAN, S. G. 1923. Pod and stem blight of soybean. Ann. Missouri Bot. Gard. 10:119-169.
10. LEHMAN, S. G., and F. A. WOLF. 1926. Soybean anthracnose. J. Agric. Res. 33:381-390.
11. LIN, Y., and L. WU. 1966. Seedborne diseases of soybean in Taiwan. II. Anthracnose. Plant Prot. Bull. (Taiwan) 8:305-317.
12. LING, L. 1940. Seedling stem blight of soybean caused by *Glomerella glycines.* Phytopathology 30:345-347.
13. SINCLAIR, J. B., and M. C. SHURTLEFF (eds.). 1975. Compendium of Soybean Diseases. American Phytopathological Society, St. Paul, Minnesota. 69 pp.
14. U. S. DEPARTMENT OF AGRICULTURE. 1960. Index of plant disease in the United States. Agriculture Handbook 165. 531 pp.
15. VON ARX, J. A. 1957. Die arten der galtun *Colletotrichum.* Phytopathol. Z. 29:413-468.